

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
14 June 2001 (14.06.2001)

PCT

(10) International Publication Number
WO 01/41704 A2(51) International Patent Classification⁷:**A61K**(74) Common Representative: MERCK & CO., INC.; 126
East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(21) International Application Number: PCT/US00/33098

(22) International Filing Date: 7 December 2000 (07.12.2000)

(25) Filing Language:

English

(26) Publication Language:

English

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS,
LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO,
NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR,
TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(30) Priority Data:

60/170,403 13 December 1999 (13.12.1999) US

(71) Applicant (for all designated States except US): MERCK
& CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway,
NJ 07065-0907 (US).(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

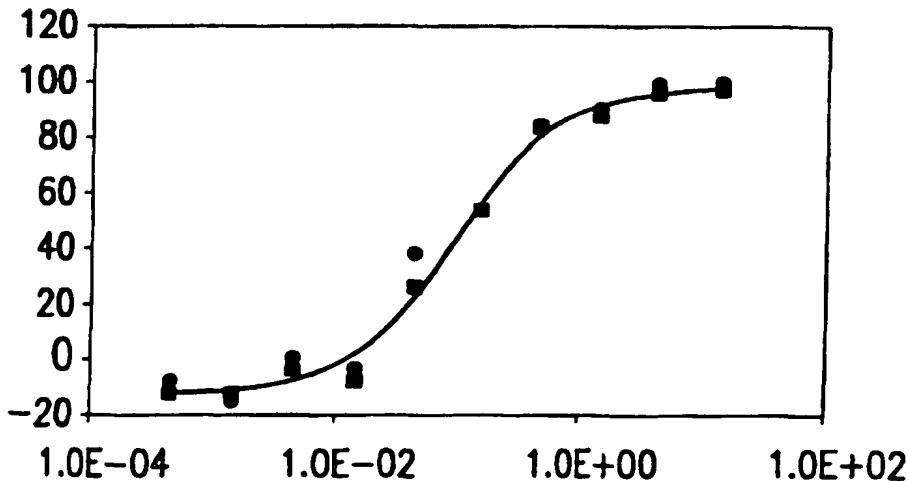
(75) Inventors/Applicants (for US only): SPARROW, Carl,
P. [US/US]; 126 East Lincoln Avenue, Rahway, NJ
07065-0907 (US). ONDEYKA, John, G. [US/US]; 126
East Lincoln Avenue, Rahway, NJ 07065-0907 (US).
SINGH, Sheo, Bux [US/US]; 126 East Lincoln Avenue,
Rahway, NJ 07065-0907 (US).

Published:

— Without international search report and to be republished
upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR THE PREVENTION AND/OR TREATMENT OF ATHEROSCLEROSIS



WO 01/41704 A2

(57) Abstract: The instant invention provides a method for raising serum HDL cholesterol levels comprising administering a therapeutically effective amount of an LXR ligand to a patient in need of such treatment. It further provides a method for using an LXR ligand to stimulate expression of the ABC1 gene. LXR ligands can be used for preventing and treating atherosclerosis and related conditions.

TITLE OF THE INVENTION

METHOD FOR THE PREVENTION AND/OR TREATMENT OF
ATHEROSCLEROSIS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to US provisional application SN 60/170,403, filed December 13, 1999, herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

10 Recent publications in *Nature Genetics*, August, 1999 (Young et al, page 316; Bodzioch et al, page 347; Brooks-Wilson et al, page 335, and Rust et al, page 352) showed that humans with mutations in the gene ABC1 have low levels of high density lipoprotein (HDL). Low HDL levels are a risk factor for atherosclerosis, myocardial infarction and related conditions such as ischemic stroke. Therefore,

15 increasing the expression of the ABC1 gene would be expected to increase HDL levels and decrease the occurrence of atherosclerosis, myocardial infarction and related conditions such as ischemic stroke. It has been reported that expression of the ABC1 gene is increased by cholesterol loading of cells (Langmann et al, *Biochem. Biophys. Res. Comm.*, 257, 29-33 (1999)). LXR α is a nuclear receptor that is required

20 for the induction of cholesterol 7 α -hydroxylase in mouse liver following cholesterol feeding (Peet et al, *Cell*, 93, 693-704 (1998)). LXR α and LXR β are activated by 22-(R)-hydroxycholesterol and other oxysterols (Janowski et al, *Proc. Natl. Acad. Sci USA*, 96, 266-271 (1999)). As part of the instant invention it was found that LXR α and/or LXR β cause the induction or regulation of ABC1 expression. We conclude that

25 small molecule ligands of LXR are useful as drugs to increase the expression of ABC1, increase levels of HDL and thereby decrease the risk of atherosclerosis, myocardial infarction and related conditions such as peripheral vascular disease and ischemic stroke.

30 SUMMARY OF THE INVENTION

One object of the instant invention is to provide a method for raising serum HDL cholesterol levels comprising administering a therapeutically effective amount of an LXR ligand to a patient in need of such treatment.

Another object is to provide a method for stimulating the expression of

35 the ABC1 gene which comprises administering an effective amount of an LXR ligand

to a patient in need of such treatment whereby the patient's serum HDL level is increased.

As a further object, methods are provided for preventing or reducing the risk of developing atherosclerosis, as well as for halting or slowing the progression of atherosclerotic disease once it has become clinically evident, comprising the administration of a prophylactically or therapeutically effective amount, as appropriate, of an LXR ligand to a patient who is at risk of developing atherosclerosis or who already has atherosclerotic disease. The method of this invention also serves to remove cholesterol from tissue deposits such as xanthomas and atherosclerotic lesions by hastening the efflux of cholesterol from cells in those lesions. Additional objects will be evident from the following detailed description.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows displacement of [³H₂]Compound A from GST-LXR α , with percent inhibition by Compound 1 at μ M concentrations. Compound 1 IC₅₀ is 80 nM (calculated K_i ~ 30 nM).

Figure 2 shows displacement of [³H₂]Compound A from GST-LXR β , with percent inhibition by Compound 1 at μ M concentrations. Compound 1 IC₅₀ is 40 nM (calculated K_i ~ 14 nM).

Figure 3 shows LXR α -GAL4 fusion protein transactivation in cultured cells by various concentrations of Compound 1.

Figure 4 shows LXR β -GAL4 fusion protein transactivation in cultured cells by various concentrations of Compound 1.

DETAILED DESCRIPTION OF THE INVENTION

Any patient desiring to increase their HDL cholesterol level may use this treatment. Particularly suitable patients in need of such treatment are those whose HDL level is below the clinically desirable level of HDL cholesterol, i.e., about 40 mg/dl in men and about 50 mg/dl in women.

Atherosclerosis encompasses vascular diseases and conditions that are recognized and understood by physicians practicing in the relevant fields of medicine. Atherosclerotic cardiovascular disease including restenosis following revascularization procedures, coronary heart disease (also known as coronary artery disease or ischemic heart disease), cerebrovascular disease including multi-infarct dementia, and peripheral vessel disease including erectile dysfunction are all clinical

manifestations of atherosclerosis and are therefore encompassed by the terms "atherosclerosis" and "atherosclerotic disease."

An LXR ligand may be administered to prevent or reduce the risk of occurrence, or recurrence where the potential exists, of a coronary heart disease event, 5 a cerebrovascular event, and/or intermittent claudication. Coronary heart disease events are intended to include CHD death, myocardial infarction (i.e., a heart attack), and coronary revascularization procedures. Cerebrovascular events are intended to include ischemic or hemorrhagic stroke (also known as cerebrovascular accidents) and transient ischemic attacks. Intermittent claudication is a clinical manifestation of 10 peripheral vessel disease. The term "atherosclerotic disease event" as used herein is intended to encompass coronary heart disease events, cerebrovascular events, and intermittent claudication. It is intended that persons who have previously experienced one or more non-fatal atherosclerotic disease events are those for whom the potential for recurrence of such an event exists.

15 Accordingly, the instant invention also provides a method for preventing or reducing the risk of a first or subsequent occurrence of an atherosclerotic disease event comprising the administration of a prophylactically effective amount of an LXR ligand to a patient at risk for such an event. The patient may already have atherosclerotic disease at the time of administration, or may be at 20 risk for developing it.

The method of this invention also serves to remove cholesterol from tissue deposits such as atherosclerotic plaques or xanthomas in a patient with atherosclerotic disease manifest by clinical signs such as angina, claudication, bruits, one that has suffered a myocardial infarction or transient ischemic attack, or one 25 diagnosed by angiography, sonography or MRI.

The term LXR includes all subtypes of this receptor and corresponding genes which encode such subtypes. Specifically LXR includes LXR α and LXR β , and a ligand of LXR should be understood to include a ligand of LXR α or LXR β . LXR α has been referred to under a variety of names and for purposes of this application 30 LXR α should be understood to mean any gene referred to as LXR α , LXRa, LXRa, LXRalphi, RLD-1, NR1H3 or a gene with homology to accession number U22662 or a protein with homology to a protein encoded by such a polynucleotide. Similarly, LXR β should be understood to include any gene referred to as LXR β , LXR β , LXRbeta, NER, NER1, UR, OR-1, R1P15, NR1H2 or a gene with homology to

accession number U07132 or a protein with homology to a protein encoded by such a polynucleotide.

The term ligand throughout this application should be understood to include an agonist, partial agonist or antagonist of LXR. The ligand may be selective

5 for LXR α or LXR β , or it may have mixed binding affinity for both LXR α and LXR β . Particularly, compounds within the scope of this invention include those which have greater selectivity as determined by binding affinity for LXR α and/or LXR β receptors than they have for each of the PPAR α , γ and δ receptors. More particularly, the compounds included within the scope of this invention have an IC₅₀ less than or

10 equal to 100nM for at least one of either the LXR α or LXR β receptors, and have an IC₅₀ equal to or greater than 1 μ M for each of the PPAR α , PPAR γ and PPAR δ receptors, and even more particularly they have an IC₅₀ equal to or greater than 10 μ M for each of the PPAR α , PPAR γ and PPAR δ receptors. For example, the selectivity of suitable LXR receptor ligands can be determined from IC₅₀ results obtained

15 employing the LXR radioligand competition scintillation proximity assays described below in the Example section, and from PPAR competition binding assays described in Berger J, et al., Novel peroxisome proliferator-activated receptor (PPAR γ) and PPAR δ ligands produce distinct biological effects, J Biol Chem 274: 6718-6725 (1999), herein incorporated by reference in its entirety.

20 The term "patient" includes mammals, especially humans, who use the instant active agents for the prevention or treatment of a medical condition.

Administering of the drug to the patient includes both self-administration and

administration to the patient by another person. The patient may be in need of treatment for an existing disease or medical condition, or may desire prophylactic

25 treatment to prevent or reduce the risk for diseases and medical conditions affected by HDL cholesterol.

The term "therapeutically effective amount" is intended to mean that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, a system, animal or human that is being sought by a researcher,

30 veterinarian, medical doctor or other clinician. The term "prophylactically effective amount" is intended to mean that amount of a pharmaceutical drug that will prevent or reduce the risk of occurrence of the biological or medical event that is sought to be prevented in a tissue, a system, animal or human by a researcher, veterinarian, medical doctor or other clinician. Particularly, the dosage amount of an LXR ligand that a

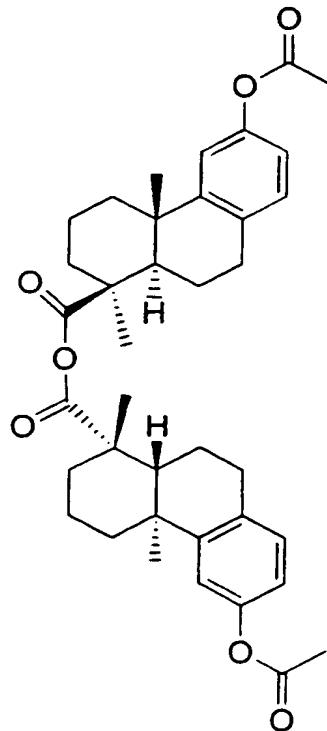
35 patient receives can be selected so as to achieve the amount of HDL cholesterol

raising desired; the dosage a patient receives may also be titrated over time in order to reach a target HDL level.

An effective amount of an LXR ligand in the method of this invention is about 0.01 mg/kg to about 140 mg/kg of body weight per day, or about 0.5 mg to 5 about 7 g per patient per day. For example, adequate elevation of HDL can be accomplished by the administration of about 0.5 mg to about 3.5 mg per patient per day.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the age, body weight, 10 general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular HDL deficiency. A consideration of these factors is well within the purview of the ordinarily skilled clinician for the purpose of determining the therapeutically effective or prophylactically effective dosage amount needed to prevent, counter, or arrest the 15 progress of the condition.

An example of an LXR ligand suitable for use in the method of this invention is represented by Compound 1 having the following structural formula



Compound 1

In the method of treatment of this invention, the LXR receptor ligands described above may be administered orally, topically, parenterally, by inhalation spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques.

The pharmaceutical compositions of this invention containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients, which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. Oral immediate-release and time-controlled release dosage forms may be employed. Tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the technique described in the U.S. Patent 4,256,108; 4,166,452; and 4,265,874 to form osmotic therapeutic tablets for controlled release.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredients is mixed with water or miscible solvents such as

propylene glycol, PEGs and ethanol, or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are 5 suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of 10 ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan 15 monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active 20 ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of 25 an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are 30 exemplified by those already mentioned above. Additional excipients, for example sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or 35 mixtures of these. Suitable emulsifying agents may be naturally-occurring

phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavouring and colouring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. Cosolvents such as ethanol, propylene glycol or polyethylene glycols may also be used. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds useful in the method of treatment of the invention may also be administered in the form of a suppository for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, gels, solutions or suspensions, etc., containing the compound of are employed. For purposes of this application, topical application shall include mouth washes and gargles. Topical formulations may generally be comprised of a pharmaceutical carrier, cosolvent, emulsifier, penetration enhancer, preservative system, and emollient.

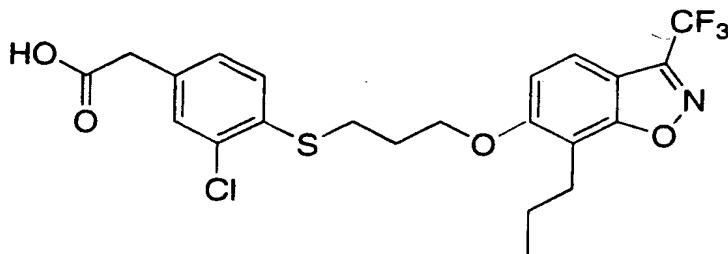
The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may contain from 0.5 mg to 5 g of active agent

compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient, typically 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 800 mg, or 1000 mg.

One or more additional active agents may be used in combination with the LXR ligands of this invention in a single dosage formulation, or may be administered to the patient in a separate dosage formulation, which allows for concurrent or sequential administration of the active agents. The additional active agent or agents can be lipid altering compounds such as HMG-CoA reductase inhibitors, or agents having other pharmaceutical activities, or agents that have both lipid-altering effects and other pharmaceutical activities. Examples of HMG-CoA reductase inhibitors include statins in their lactonized or dihydroxy open acid forms and pharmaceutically acceptable salts and esters thereof, including but not limited to lovastatin (see US Patent No. 4,342,767); simvastatin (see US Patent No. 4,444,784), pravastatin, particularly the sodium salt thereof (see US Patent No. 4,346,227); fluvastatin particularly the sodium salt thereof (see US Patent No. 5,354,772); atorvastatin, particularly the calcium salt thereof (see US Patent No. 5,273,995); cerivastatin, particularly the sodium salt thereof (see US Patent No. 5,177,080), and nisvastatin also referred to as NK-104 (see PCT international publication number WO 97/23200). Additional active agents which may be employed in combination with an LXR ligand include but are not limited to HMG-CoA synthase inhibitors; squalene epoxidase inhibitors; squalene synthetase inhibitors (also known as squalene synthase inhibitors), acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibitors including selective inhibitors of ACAT-1 or ACAT-2 as well as dual inhibitors of ACAT1 and -2; microsomal triglyceride transfer protein (MTP) inhibitors; probucol; niacin; cholesterol absorption inhibitors such as SCH-58235; bile acid sequestrants; LDL (low density lipoprotein) receptor inducers; platelet aggregation inhibitors, for example glycoprotein IIb/IIIa fibrinogen receptor antagonists and aspirin; human peroxisome proliferator activated receptor gamma (PPAR γ) agonists including the compounds commonly referred to as glitazones for example troglitazone, pioglitazone and rosiglitazone and, including those compounds included within the structural class known as thiazolidinediones as well as those PPAR γ agonists outside the thiazolidinedione structural class; PPAR α agonists such as clofibrate, fenofibrate including micronized fenofibrate, and gemfibrozil; PPAR dual α/γ agonists; vitamin

B₆ (also known as pyridoxine) and the pharmaceutically acceptable salts thereof such as the HCl salt; vitamin B₁₂ (also known as cyanocobalamin); folic acid or a pharmaceutically acceptable salt or ester thereof such as the sodium salt and the methylglucamine salt; anti-oxidant vitamins such as vitamin C and E and beta carotene; beta-blockers; angiotensin II antagonists such as losartan; angiotensin converting enzyme inhibitors such as enalapril and captopril; calcium channel blockers such as nifedipine and diltiazem; endothelin antagonists; agents other than LXR ligands that enhance ABC1 gene expression; bisphosphonate compounds such as alendronate sodium; and cyclooxygenase-2 inhibitors such as rofecoxib and celecoxib.

Compound A is used in the following assays and has the following structural formula:



15

Compound A

Compound A and related compounds are disclosed along with methods for making them in WO97/28137 herein incorporated by reference in its entirety (US Serial No. 08/791211, filed January 31, 1997).

20

EXAMPLE 1

**Radioligand Competition Binding Scintillation Proximity Assays:
Preparation of Recombinant Human LXR α and LXR β :**

25 Human LXR α and LXR β were expressed as GST-fusion proteins in *E. coli*. The ligand binding domain cDNAs for human LXR α (amino acids 164-447) and human LXR β (amino acids 149-455) were subcloned into the pGEX-KT expression vector (Pharmacia). *E. coli* containing the respective plasmids were propagated,

induced, and harvested by centrifugation. The resuspended pellet was broken in a French press and debris was removed by centrifugation. Recombinant human LXR receptors were purified by affinity chromatography on glutathione sepharose and receptor was eluted with glutathione. Glycerol was added to a final concentration of 5 50% to stabilize the receptor and aliquots were stored at -80 °C.

Binding to LXR α :

For each assay, an aliquot of human GST-LXR α receptor was incubated in a final volume of 100 μ l SPA buffer (10 mM Tris, pH 7.2, 1 mM EDTA, 10% glycerol, 10 mM Na molybdate, 1 mM dithiothreitol, and 2 μ g/ml benzamidine) 10 containing 1.25 mg/ml yttrium silicate protein A coated SPA beads (Amersham Pharmacia Biotech, Inc.), 8.3 μ g/ml anti-GST antibody (Amersham Pharmacia Biotech, Inc.) 0.1% non-fat dry milk and 25 nM [3 H₂]Compound A (13.4 Ci/mmole), \pm test compound. After incubation for ~16 h at 15°C with shaking, the 15 assay plates were counted in a Packard Topcount. In this assay the K_d for Compound A for LXR α is \approx 15 nM.

Binding to LXR β :

For each assay, an aliquot of human GST-LXR β ligand binding 20 domain receptor was incubated in a final volume of 100 μ l SPA buffer (10 mM Tris, pH 7.2, 1 mM EDTA, 10% glycerol, 10 mM Na molybdate, 1 mM dithiothreitol, and 2 μ g/ml benzamidine) containing 1.25 mg/ml yttrium silicate protein A coated SPA beads (Amersham Pharmacia Biotech, Inc.), 8.3 μ g/ml anti-GST antibody (Amersham Pharmacia Biotech, Inc.) 0.1% non-fat dry milk and 25 nM [3 H₂]Compound A (13.4 Ci/mmole), \pm test compound. After incubation for ~16 h at 15°C with shaking, the 25 assay plates were counted in a Packard Topcount. In this assay the K_d for Compound A for LXR β is \approx 10 nM.

30 Results

Compound I is a ligand for human LXR α and human LXR β , having an IC₅₀ = 80nM for the LXR α receptor, and an IC₅₀ = 40nM for the LXR β receptor, as shown in Figures 1 and 2. Compound I has an IC₅₀ greater than 10 μ M in binding assays for human PPAR γ , PPAR δ and PPAR α .

EXAMPLE 2

Transactivation Assay

Plasmids

5 Expression constructs were prepared by inserting the ligand binding domain (LBD) of human LXR α and LXR β cDNAs adjacent to the yeast GAL4 transcription factor DNA binding domain (DBD) in the mammalian expression vector pcDNA3 to create pcDNA3-LXR α /GAL4 and pcDNA3-LXR β /GAL4, respectively. The GAL4-responsive reporter construct, pUAS(5X)-tk-luc, contained 5 copies of the
10 GAL4 response element placed adjacent to the thymidine kinase minimal promoter and the luciferase reporter gene. The transfection control vector, pEGFP-N1, contained the Green Fluorescence Protein (GFP) gene under the regulation of the cytomegalovirus promoter.

15 Assay HEK-293 cells were seeded at 40,000 cells/well in 96 well plates in Dulbecco's modified Eagle medium (high glucose) containing 10% charcoal stripped fetal calf serum, 100 units/ml Penicillin G and 100 μ g/ml Streptomycin sulfate at 37°C in a humidified atmosphere of 5% CO₂. After 24 h, transfections were performed with
20 Lipofectamine (Gibco-BRL, Gaithersburg, MD) according to the instructions of the manufacturer. In general, transfection mixes contained 0.002 μ g of LXR α /GAL4 or LXR β /GAL4 chimeric expression vectors, 0.02 μ g of reporter vector pUAS(5X)-tk-luc and 0.034 μ g of pEGFP-N1 vector as an internal control of transfection efficiency. Compounds were characterized by incubation with transfected cells for 48h across a
25 range of concentrations. Cell lysates were prepared from washed cells using Cell Lysis Buffer (Promega) according to the manufacturer's directions. Luciferase activity in cell extracts was determined using Luciferase Assay Buffer (Promega) in a ML3000 luminometer (Dynatech Laboratories). GFP expression was determined using the Tecan Spectrofluor Plus at excitation wavelength of 485nm and emission at
30 535nm. Luciferase activity was normalized to GFP expression to account for any variation in efficiency of transfection.

Results with Compound 1 for LXR α transactivation are shown in Figure 3, and results for LXR β transactivation are shown in Figure 4.

Induction of ABC1 mRNA levels

Cultured human THP-1 cells were stimulated to differentiate into macrophages by incubation with 100 nM tetradecanoyl phorbol acetate for three days. All cell culture incubations were performed at 37°C under 95% air/5% carbon dioxide using culture conditions as recommended by ATCC. After differentiation, the THP-1 macrophages were incubated with the test LXR agonist. After 6 hours at 37°C, the cells were harvested and total RNA prepared using the phenol/guanidine isothiocyanate method as supplied and described by Molecular Research Center, Inc. (TRI REAGENT® Cat. No. TR 118). ABC1 mRNA levels in the total RNA were measured using the TaqMan® mRNA quantitation system, following protocols published by the manufacturer (Perkin-Elmer). The oligonucleotide PCR primers used to detect ABC1 were:

GAGGCTCCCGAGTTGTTG and GTATAAAAGAAGCCTCCGAGCATC
The oligonucleotide probe used was:

15 6FAM-AAACTTAAACAAATCCATTGTGGCTGCCTGT-TAMRA

ABC1 mRNA levels in each sample were normalized to the mRNA levels for the 23 kDa highly basic protein. The oligonucleotide PCR primers used to detect the 23 kDa highly basic protein were:

GCTGGAAGTACCAGGCAGTGA and ACCGGTAGTGGATCTGGCTTT

20 The oligonucleotide probe used was:

VIC- TCTTCCTCTCCTCCAGGGTGGCT-TAMRA

The results from this experiment for Compound 1 and 22-(R)-hydroxycholesterol are as follows:

Compound	Fold Induction of ABC1 mRNA (Mean \pm SEM)	P Value vs DMSO Control
15 μ M 22-(R)-hydroxycholesterol	6.7 \pm 1.2	0.008
0.10 μ M Compound 1	6.9 \pm 0.6	0.0009

25

EXAMPLE 4

Preparation of Compound 1

Podocarpic acid (550 mg) was dissolved in 2 ml of acetic anhydride in a 10 ml flask and heated to reflux (150 °C) for 30 minutes and cooled. The reaction was analyzed by HPLC. The major product was the mixed anhydride and about 1% of the reaction mixture was the acetate dimer Compound 1. The solvent was blown off under nitrogen and the resultant oil was charged to a 200 cc Sephadex LH20 column in MeOH (methanol). Compound 1 eluted in cuts 75-80 (2 ml each, 0.8 cv). The mixed anhydride eluted in cuts 80-100. Cuts 75-80 were dried down and dissolved in 300 μ l CH₃CN and loaded on a semi-preparative Zorbax RX-C8 column. The column was eluted with a 40-min gradient of 50 to 90% aqueous CH₃CN at 4 mL per min. One-minute fractions were collected. Compound 1 eluted at 35 min. The pooled fractions gave 0.7 mg of Compound 1. Mass spectral analysis of this compound gave a molecular weight of 614 amu and molecular formula of C₃₈H₄₆O₇.

15 Mass spectral data: Found: 632.3620; Calculated: 632.3587; Formula: C₃₈H₅₀NO₇; Assignment: [M⁺NH₄].

¹H NMR data: (δ , 500 MHz, CDCl₃): δ 1.14(1H, dt, 13.5, 4.0 Hz), 1.20(3H,s), 1.39(3H,s), 1.44(1H,dt, 13.5, 4.0 Hz), 1.63(1H, d, 12.5), 1.67(1H, m), 2.0(1H, d, 14.0 Hz), 2.05(1H,m), 2.2(1H, dd, 13.5, 6.0 Hz), 2.25(1H,d, 12 Hz), 2.28(3H,s), 2.30(1H,d, 13.5 Hz), 2.80(1H,ddd, 13.0, 12.5, 6.5 Hz), 2.95(1H, dd, 16.5, 5.0 Hz), 6.83(1H, dd, 8.0, 2.0 Hz), 6.96(1H,d, 2.0Hz), 7.05(1H,d, 8.0 Hz).

Equipment: Mass spectra were recorded on an LCQ (LC-MS-ESI, Liquid chromatography-Electrospray ionization) and exact mass measurements were recorded on a Finnigan NewStar FTMS mass spectrometer. ^1H spectra were recorded in either CDCl_3 or CD_3OD on a Varian Unity 500 NMR Spectrometer operating at 500 MHz for ^1H . Chemical shifts are given in ppm relative to tetramethylsilane (TMS) at zero ppm using the respective solvent peaks as an internal standard.

30 While the invention has been described and illustrated with reference
to certain particular embodiments thereof, those skilled in the art will appreciate that
various changes, modifications and substitutions can be made therein without
departing from the spirit and scope of the invention. For example, effective dosages
35 other than the particular dosages as set forth herein above may be applicable as a

consequence of variations in the responsiveness of the mammal being treated for any of the indications for the active agents used in the instant invention as indicated above. Likewise, the specific pharmacological responses observed may vary according to and depending upon the particular active compound selected or whether 5 there are present pharmaceutical carriers, as well as the type of formulation employed, and such expected variations or differences in the results are contemplated in accordance with the objects and practices of the present invention. It is intended, therefore, that the invention be defined by the scope of the claims which follow and that such claims be interpreted as broadly as is reasonable.

10

WHAT IS CLAIMED:

1. A method for raising serum HDL cholesterol levels comprising administering an effective HDL-raising amount of an LXR receptor ligand to a patient in need of such treatment.
2. The method of Claim 1, wherein the LXR receptor is an LXRx receptor.
- 10 3. The method of Claim 1, wherein the LXR receptor is an LXRB receptor.
4. The method of Claim 1, wherein the ligand is an agonist.
- 15 5. The method of Claim 1, wherein the ligand is an antagonist.
6. The method of Claim 1, wherein the ligand is a partial agonist.
7. The method of Claim 2, wherein the ligand is an agonist.
- 20 8. The method of Claim 2, wherein the ligand is an antagonist.
9. The method of Claim 2, wherein the ligand is a partial agonist.
10. The method of Claim 3, wherein the ligand is an agonist.
- 25 11. The method of Claim 3, wherein the ligand is an antagonist.
12. The method of Claim 3, wherein the ligand is a partial agonist.
- 30 13. The method of Claim 1 wherein the LXR ligand binds with greater affinity to an LXR receptor than to a PPAR receptor.
14. The method of Claim 13 wherein the LXR ligand has an IC50 less than or equal to 100 nM for at least one of an LXR receptor selected from LXRx

and LXR β , and an IC₅₀ equal to or greater than 1 μ M for each of the PPAR α , PPAR γ and PPAR δ receptors.

15. The method of Claim 14 wherein the LXR ligand has an IC₅₀
5 equal to or greater than 10 μ M for each of the PPAR α , PPAR γ and PPAR δ receptors.

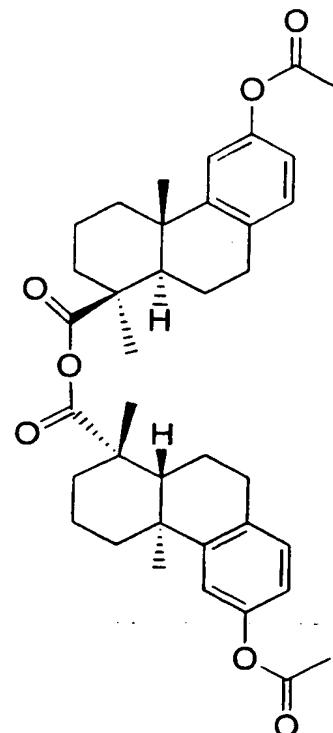
16. A method for preventing or reducing the risk of developing
atherosclerotic disease comprising administering an HDL-raising amount of an LXR
receptor ligand to a patient in need of such treatment.

10 17. A method for treating atherosclerotic disease comprising
administering an HDL-raising amount of an LXR receptor ligand to a patient in need
of such treatment.

15 18. A method for preventing or reducing the risk of occurrence or
recurrence of an atherosclerotic disease event comprising administering an HDL-
raising amount of an LXR receptor ligand to a patient in need of such treatment.

19. A method for stimulating the expression of the ABC1 gene and
20 thereby raising serum HDL cholesterol levels comprising administering an LXR
ligand in an amount capable of stimulating expression of the ABC1 gene to a patient
in need of such treatment.

20. A compound having the following structural formula



1/4

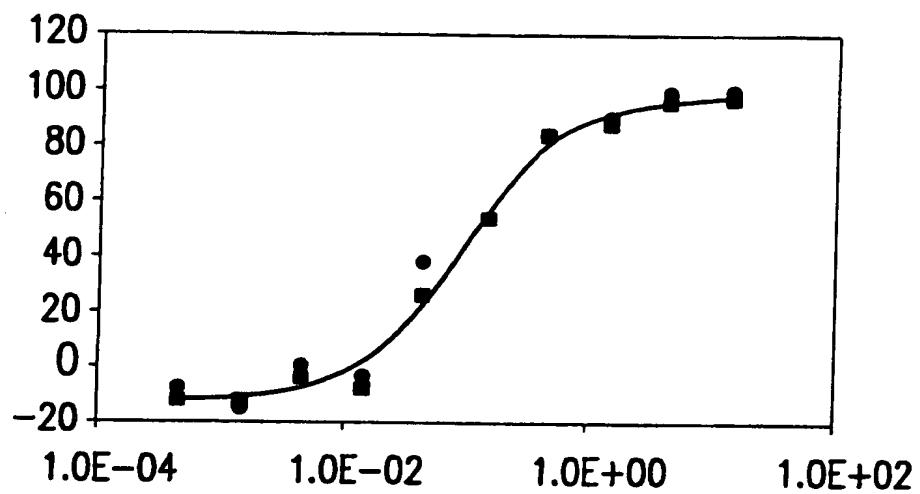


FIG. 1

2/4

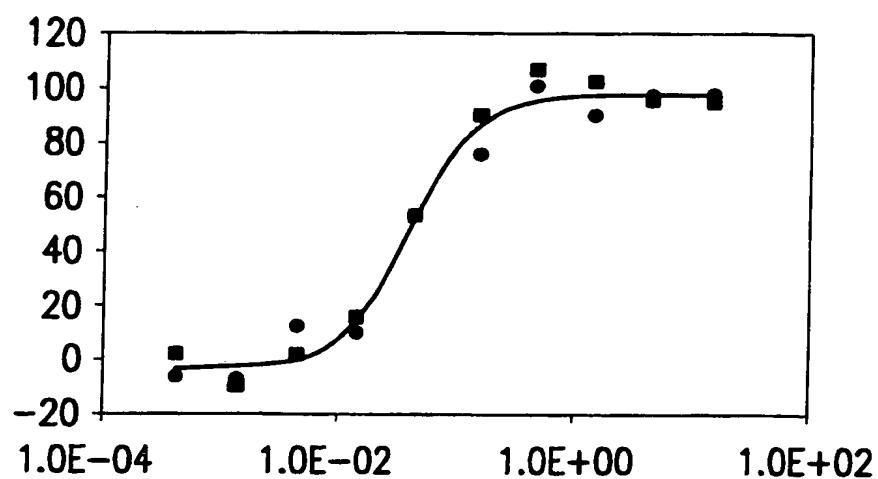


FIG.2

3/4

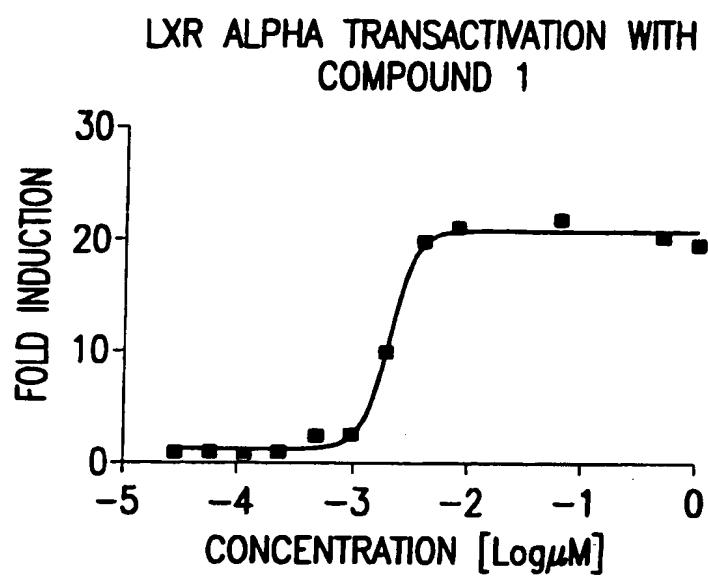


FIG.3

4/4

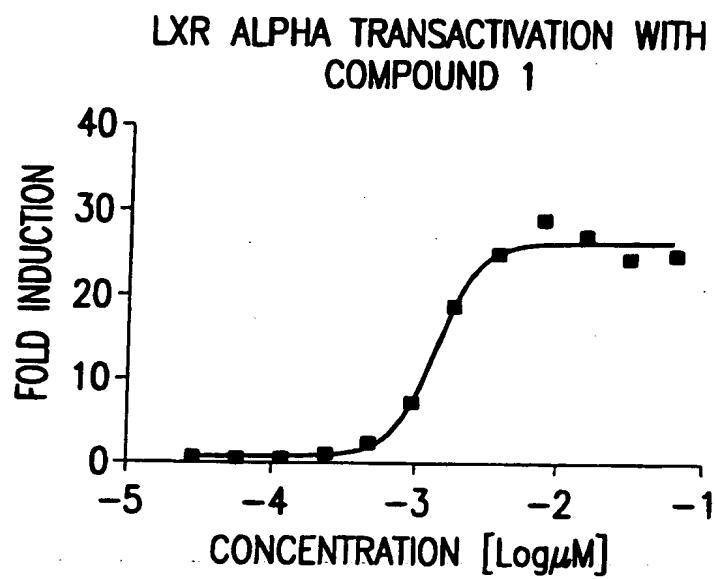


FIG.4

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 June 2001 (14.06.2001)

PCT

(10) International Publication Number
WO 01/41704 A3

(51) International Patent Classification: A61K 31/21. (31/35, 31/19) (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(21) International Application Number: PCT/US00/33098

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(22) International Filing Date: 7 December 2000 (07.12.2000)

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/170,403 13 December 1999 (13.12.1999) US

Published:

— with international search report

(71) Applicant (*for all designated States except US*): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(88) Date of publication of the international search report:

6 December 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): SPARROW, Carl, P. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). ONDEYKA, John, G. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US). SINGH, Sheo, Bux [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

WO 01/41704 A3

(54) Title: METHOD FOR THE PREVENTION AND/OR TREATMENT OF ATHEROSCLEROSIS

(57) Abstract: The instant invention provides a method for raising serum HDL cholesterol levels comprising administering a therapeutically effective amount of an LXR ligand to a patient in need of such treatment. It further provides a method for using an LXR ligand to stimulate expression of the ABC1 gene. LXR ligands can be used for preventing and treating atherosclerosis and related conditions.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/33098

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/21, 31/35, 31/19
US CL : 514/510, 460, 569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/510, 460, 569

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: compounds and therapeutic methods, podocarpic acid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,444,784 A (HOFFMAN et al) 24 April 1984, see entire document, esp columns 1 and 13	1-19
Y	WINDHOLZ et al "Podocarpic Acid. 1,2,3,4,4a,9, 10,10a-Octahydro-6-hydroxy-1,4a-dimethyl-1-phenanthrenecarboxylic acid; 12-hydroxypodocarpa-8,11,13-trein-16-oic acid". In: Merck Index, Eds. Merck Dohm & Sharp, Rahway, N.J., 1983, see abstract No. 7411	20
Y	Database CA on STN, (Columbus, Ohio, USA), AN 180872, KAGAWA et al, "Eicosapolyenoic acids of serum lipids of Japanese Islanders with low incidence of cardiovascular diseases", J. Nutr. Sci. Vitaminol., 28(4), 1982, see entire abstract.	1-20

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:		
"A" document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but aimed to understand the principle or theory underlying the invention
"B" earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"a"	document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

10 JULY 2001

Date of mailing of the international search report

02 AUG 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-9250

Authorized officer
Russell Travers
RUSSELL TRAVERS
Telephone No. (703) 305-1235